P&S Accelerating Genomics

Lecture 3:
Introduction to Sequencing

Dr. Mohammed Alser


@mealser

ETH Zurich

Fall 2022

3 November 2022

SAFARI

ETH zürich

Agenda for Today

- What is Genome Analysis?
- What is Intelligent Genome Analysis?
- How we Analyze Genome?

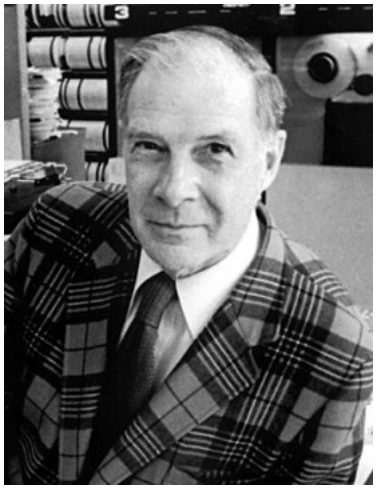
Agenda for Today

- **What is Genome Analysis?**
- What is Intelligent Genome Analysis?
- How we Analyze Genome?

What is Data Analysis?

"The purpose of computing is [to gain] insight, not numbers"

Richard Hamming



What is Genome Analysis?

https://onlinelearning.hms.harvard.edu/hmx/courses/genetic-testing/
https://www.nature.com/subjects/genomic-analysis

What is Genome Analysis?

nature research

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nature › subjects › genomic analysis

Genomic analysis

Atom RSS Feed

Genomic analysis is the identification, measurement or comparison of genomic features such as DNA sequence, structural variation, gene expression, or regulatory and functional element annotation at a genomic scale. Methods for genomic analysis typically require high-throughput sequencing or microarray hybridization and bioinformatics.

https://onlinelearning.hms.harvard.edu/hmx/courses/genetic-testing/
https://www.nature.com/subjects/genomic-analysis

DNA Testing

Health + Ancestry Service

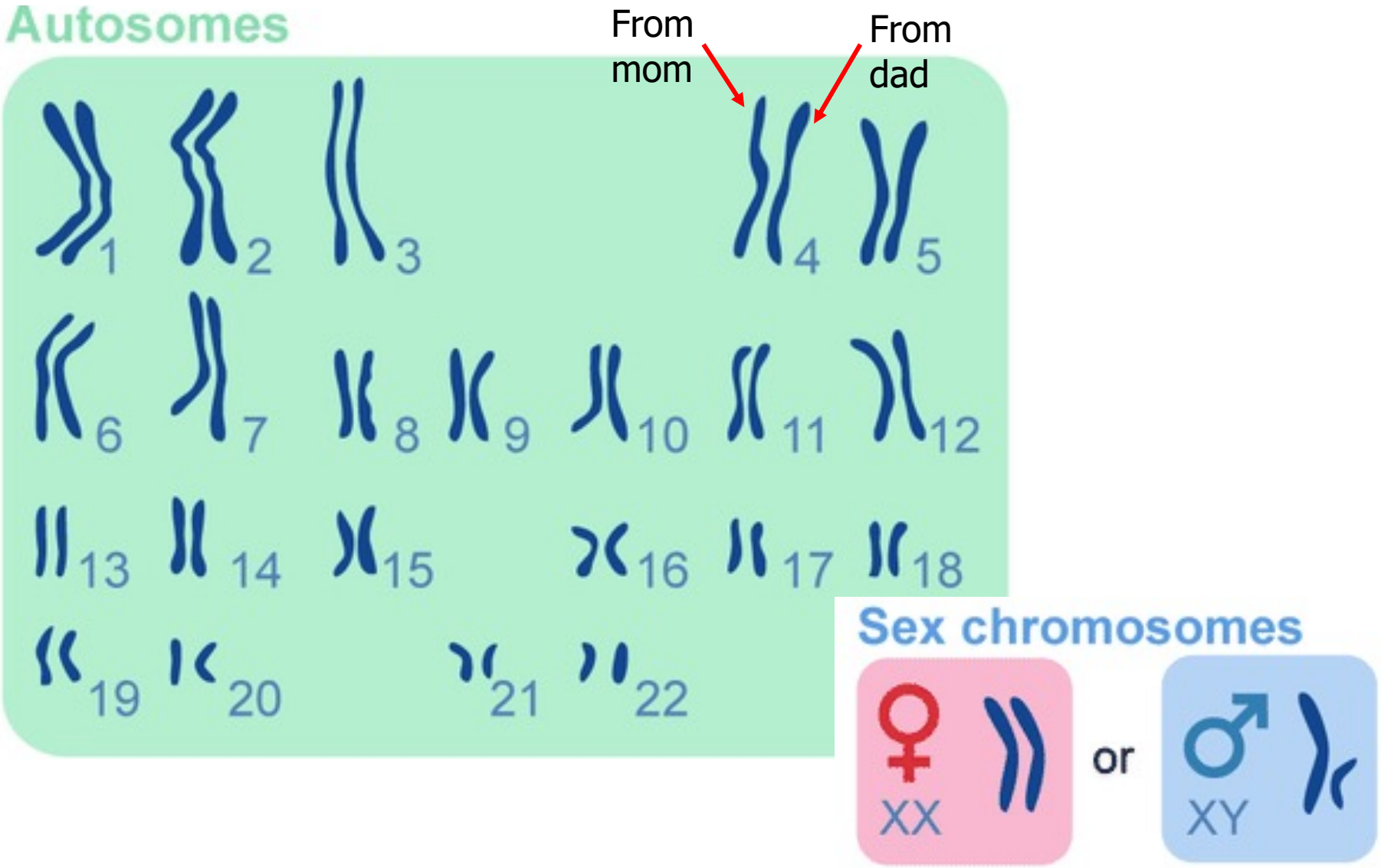
$199

- Includes everything in Ancestry + Traits Service

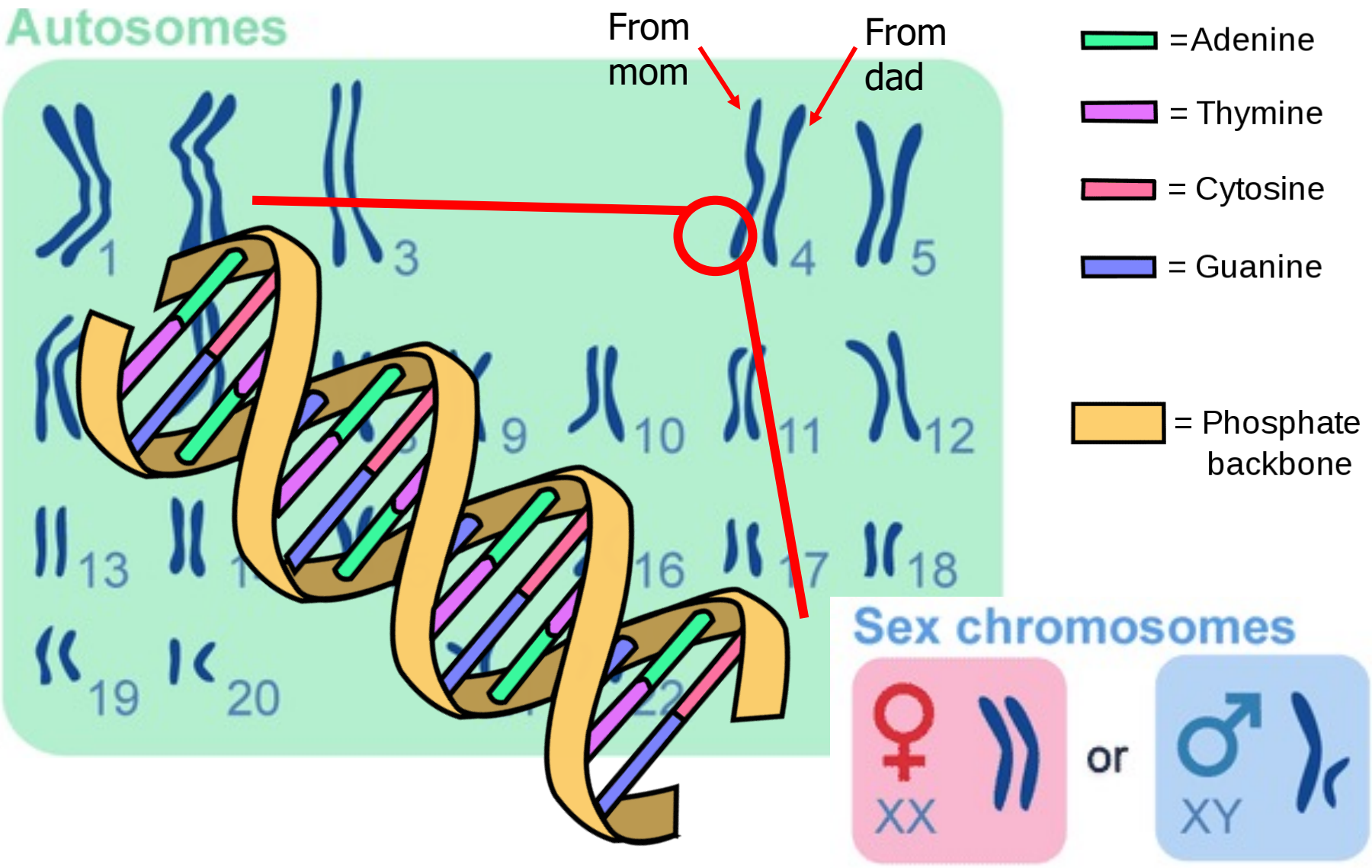
PLUS

- 10+ Health Predisposition reports*
- 5+ Wellness reports
- 40+ Carrier Status reports*

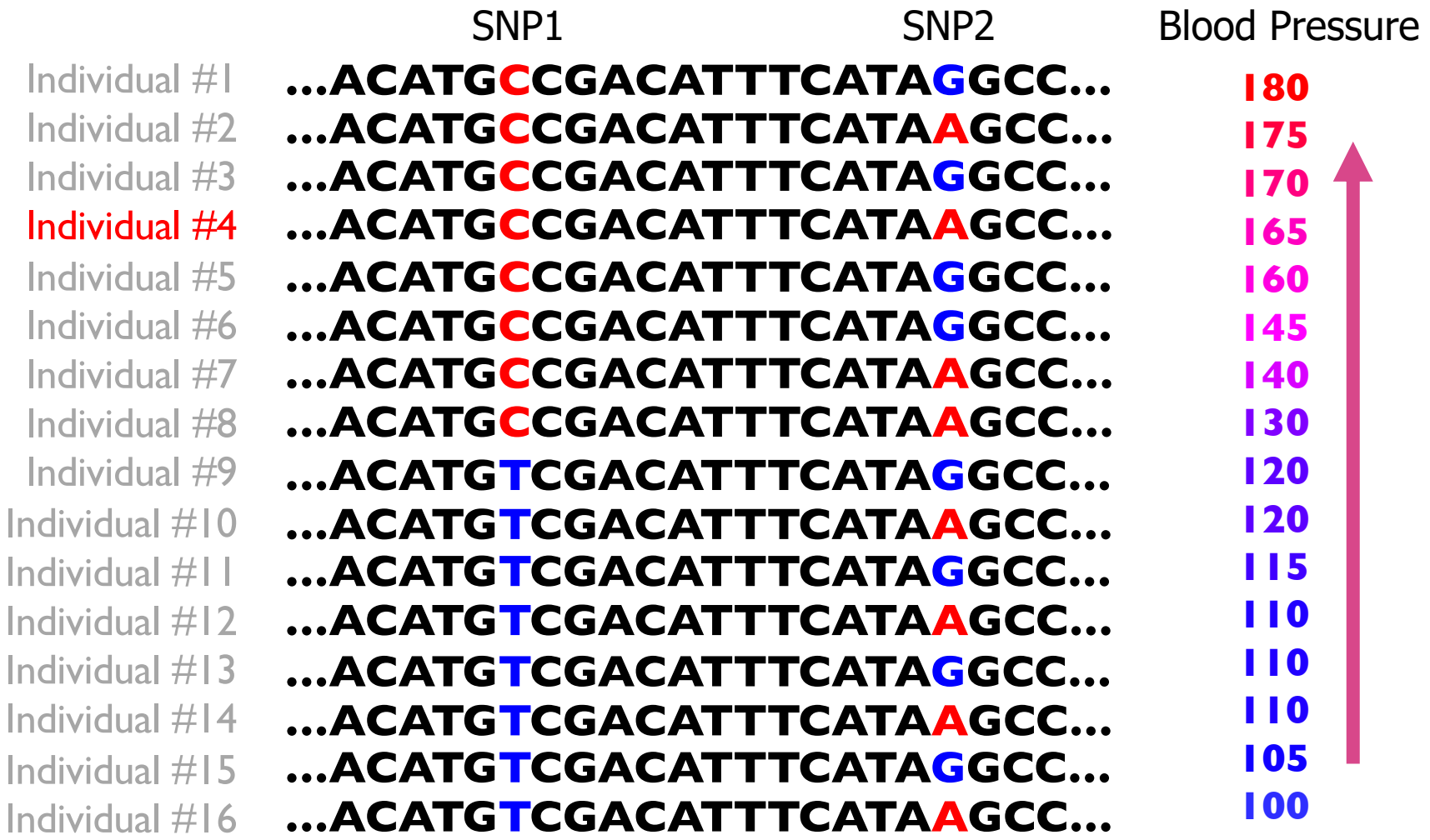
Human Chromosomes (23 Pairs)



Human Chromosomes (23 Pairs)



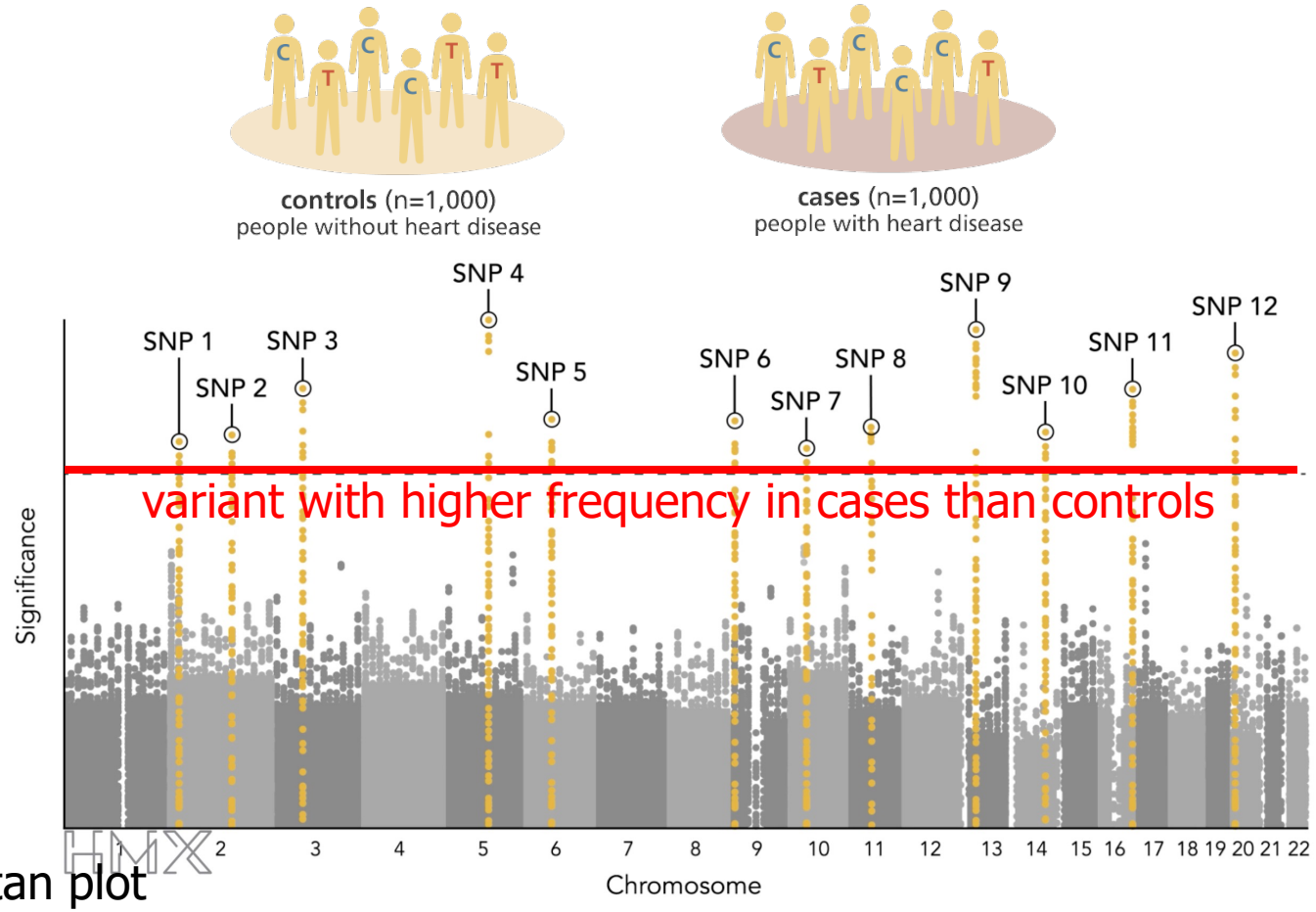
Finding SNPs Associated with Complex Trait



SNP: single nucleotide polymorphism

Genome-Wide Association Study (GWAS)

- Detecting genetic variants associated with phenotypes using two groups of people.



Manhattan plot

Similar Association Studies

PERSPECTIVE
https://doi.org/10.1038/s41588-019-0385-z
nature genetics

Opportunities and challenges for transcriptome-wide association studies

Michael Wainberg[1], Nasa Sinnott-Armstrong[2], Nicholas Mancuso[3], Alvaro N. Barbeira[4], David A. Knowles[5,6], David Golan[2], Raili Ermel[7], Arno Ruusalepp[7,8], Thomas Quertermous[9], Ke Hao[10], Johan L. M. Björkegren[8,10,11,12*], Hae Kyung Im[4*], Bogdan Pasaniuc[3,13,14*], Manuel A. Rivas[15*] and Anshul Kundaje[1,2*]

Transcriptome-wide association studies (TWAS) integrate genome-wide association studies (GWAS) and gene expression datasets to identify gene–trait associations. In this Perspective, we explore properties of TWAS as a potential approach to prioritize causal genes at GWAS loci, by using simulations and case studies of literature-curated candidate causal genes for schizophrenia, low-density-lipoprotein cholesterol and Crohn's disease. We explore risk loci where TWAS accurately prioritizes the likely causal gene as well as loci where TWAS prioritizes multiple genes, some likely to be non-causal, owing to sharing of expression quantitative trait loci (eQTL). TWAS is especially prone to spurious prioritization with expression data from non-trait-related tissues or cell types, owing to substantial cross-cell-type variation in expression levels and eQTL strengths. Nonetheless, TWAS prioritizes candidate causal genes more accurately than simple baselines. We suggest best practices for causal-gene prioritization with TWAS and discuss future opportunities for improvement. Our results showcase the strengths and limitations of using eQTL datasets to determine causal genes at GWAS loci.

Wainberg+, "Opportunities and challenges for transcriptome-wide association studies", *Nature genetics,* 2019.

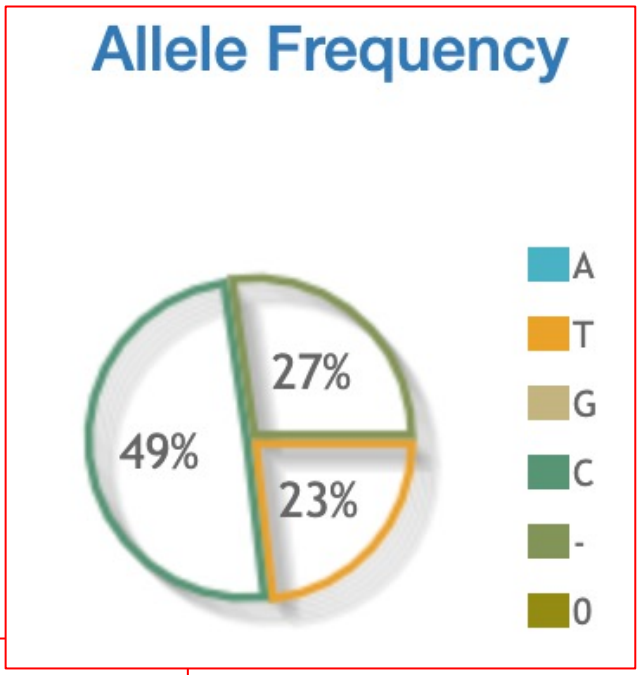
SNPs and Personalized Medicine

openSNP Search

SNP rs12979860

Basic Information

Name	rs12979860
Chromosome	19
Position	39248147
Weight of evidence	926



Links to SNPedia

Title	Summary
rs12979860 T/T	~20-25% of such hepatitis c patients respond to treatment
rs12979860 C/C	~80% of such hepatitis c patients respond to treatment
rs12979860 C/T	~20-40% of such hepatitis c patients respond to treatment

Personalized Medicine for Critically Ill Infants

- rWGS can be performed in 2-day (costly) or 5-day time to interpretation.
- Diagnostic rWGS for infants
  - Avoids morbidity
  - Reduces hospital stay length by 6%-69%
  - Reduces inpatient cost by $800,000-$2,000,000.

Article | Open Access | Published: 04 April 2018

Rapid whole-genome sequencing decreases infant morbidity and cost of hospitalization

Lauge Farnaes, Amber Hildreth, Nathaly M. Sweeney, Michelle M. Clark, S
Chowdhury, Shareef Nahas, Julie A. Cakici, Wendy Benson, Robert H. Kap
Richard Kronick, Matthew N. Bainbridge, Jennifer Friedman, Jeffrey J. Go
Ding, Narayanan Veeraraghavan, David Dimmock & Stephen F. Kingsmore

npj Genomic Medicine **3**, Article number: 10 (2018) | Cite this article

Article | Open Access | Published: 05 May 2020

Clinical utility of 24-h rapid trio-exome sequencing for critically ill infants

Huijun Wang, Yanyan Qian, Yulan Lu, Qian Qin, Guoping Lu, Guoqiang Cheng, Ping Zhang, Lin Yang, Bingbing Wu ✉ & Wenhao Zhou ✉

npj Genomic Medicine **5**, Article number: 20 (2020) | Cite this article

Farnaes+, "Rapid whole-genome sequencing decreases infant morbidity and cost of hospitalization", NPJ Genomic Medicine, 2018

Personalized Medicine in UK

"From 2019, all seriously ill children in UK will be offered whole genome sequencing as part of their care"

NHS
National Institute for Health Research

Much Larger Structural Variations!

AUTISM
Weiss, *N Eng J Med* 2008
Deletion of 593 kb

SCHIZOPHRENIA
McCarthy, *Nat Genet* 2009
Duplication of 593 kb

OBESITY
Walters, *Nature* 2010
Deletion of 593 kb

UNDERWEIGHT
Jacquemont, *Nature* 2011
Duplication of 593 kb

Deletion in the short arm
of chromosome 16 (16p11.2)

Duplication in the short arm
of chromosome 16 (16p11.2)

Recommended Reading

nature reviews genetics

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Review Article | Published: 15 November 2019

Structural variation in the sequencing era

Steve S. Ho, Alexander E. Urban & Ryan E. Mills ✉

Nature Reviews Genetics **21**, 171–189(2020) | Cite this article

15k Accesses | **16** Citations | **309** Altmetric | Metrics

Ho+, "Structural variation in the sequencing era", Nature Reviews Genetics, 2020

Agenda for Today

- What is Genome Analysis?
- **What is Intelligent Genome Analysis?**
- How we Analyze Genome?

What is Intelligent Genome Analysis?

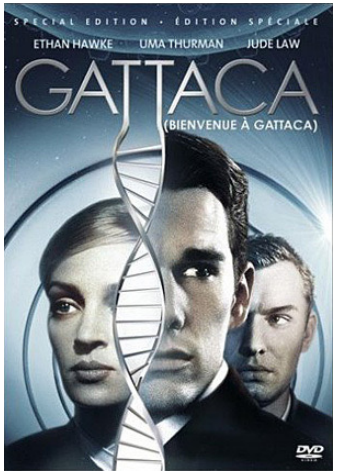
- Fast genome analysis — Bandwidth
  - *Real-time analysis*
- Using intelligent architectures — Energy-efficiency & Latency
  - *Specialized HW with less data movement*
- DNA is a valuable asset — Privacy
  - *Controlled-access analysis*
- Population-scale genome analysis — Scalability
  - *Sequence anywhere at large scale!*
- Avoiding erroneous analysis — Accuracy
  - *E.g., your father is not your father*

Does intelligent genome analysis really matter?

Fast Genome Analysis?

- **Fast** genome analysis in mere seconds using limited computational resources (i.e., personal computer or small hardware).

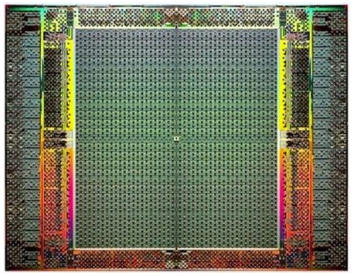
1997



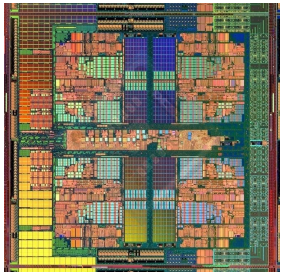
2015

Intelligent Architecture?

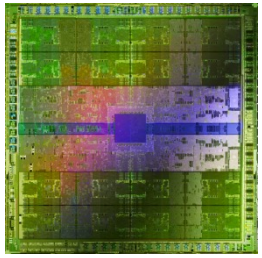
FPGAs



Modern systems

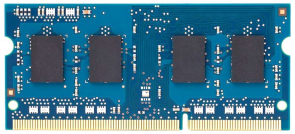


Heterogeneous Processors and Accelerators



(General Purpose) GPUs

Hybrid Main Memory



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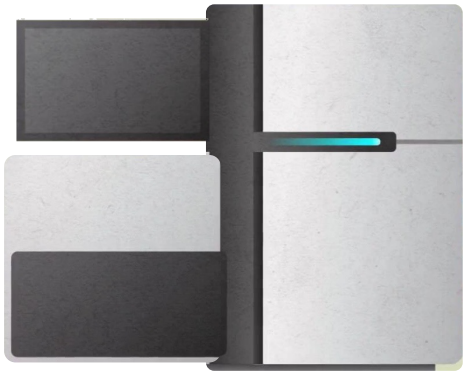
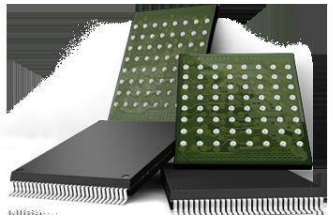
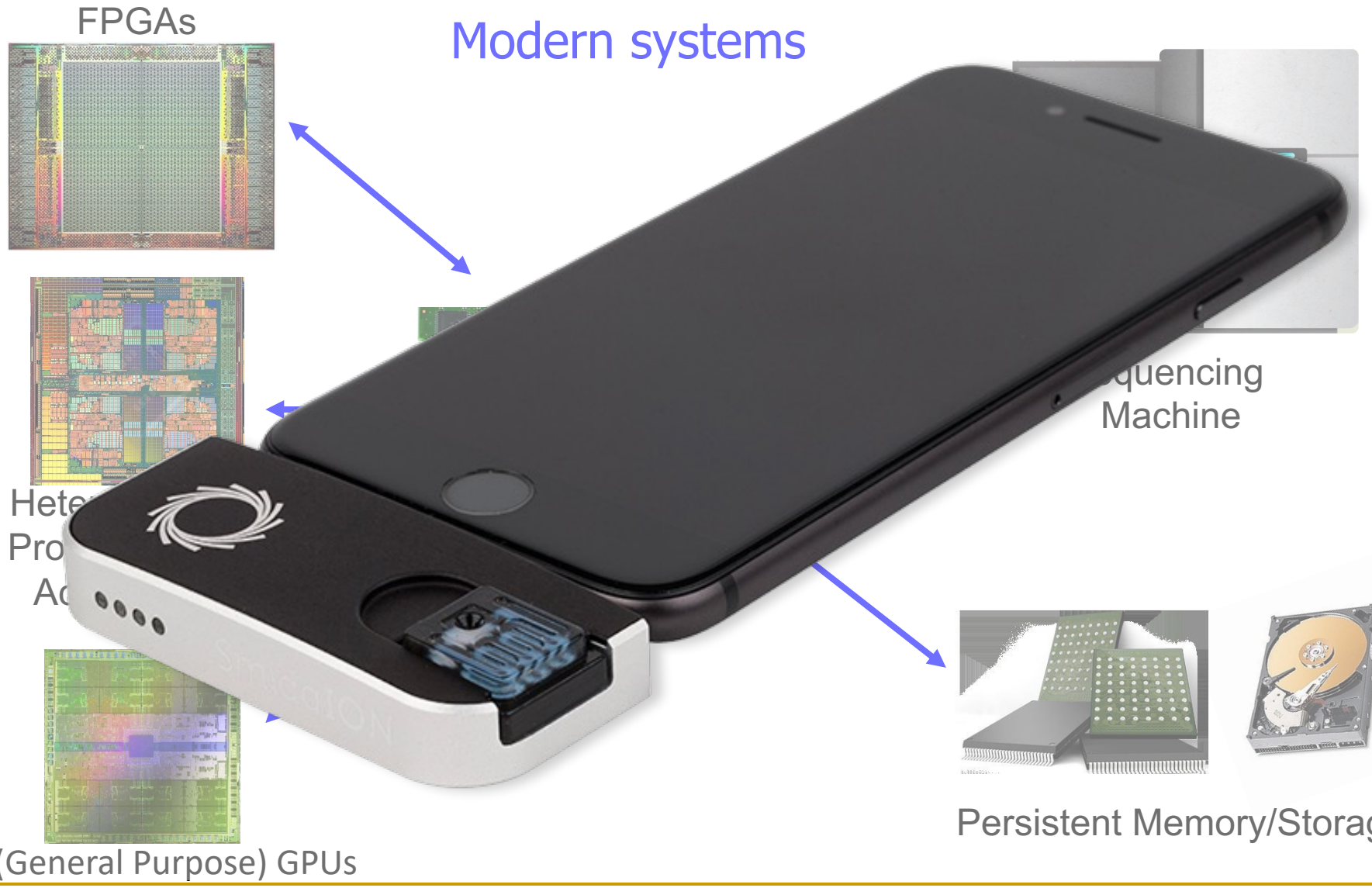


Sequencing Machine



Persistent Memory/Storage

Intelligent Architecture?



https://nanoporetech.com/products/smidgion

Privacy-Preserving Genome Analysis?

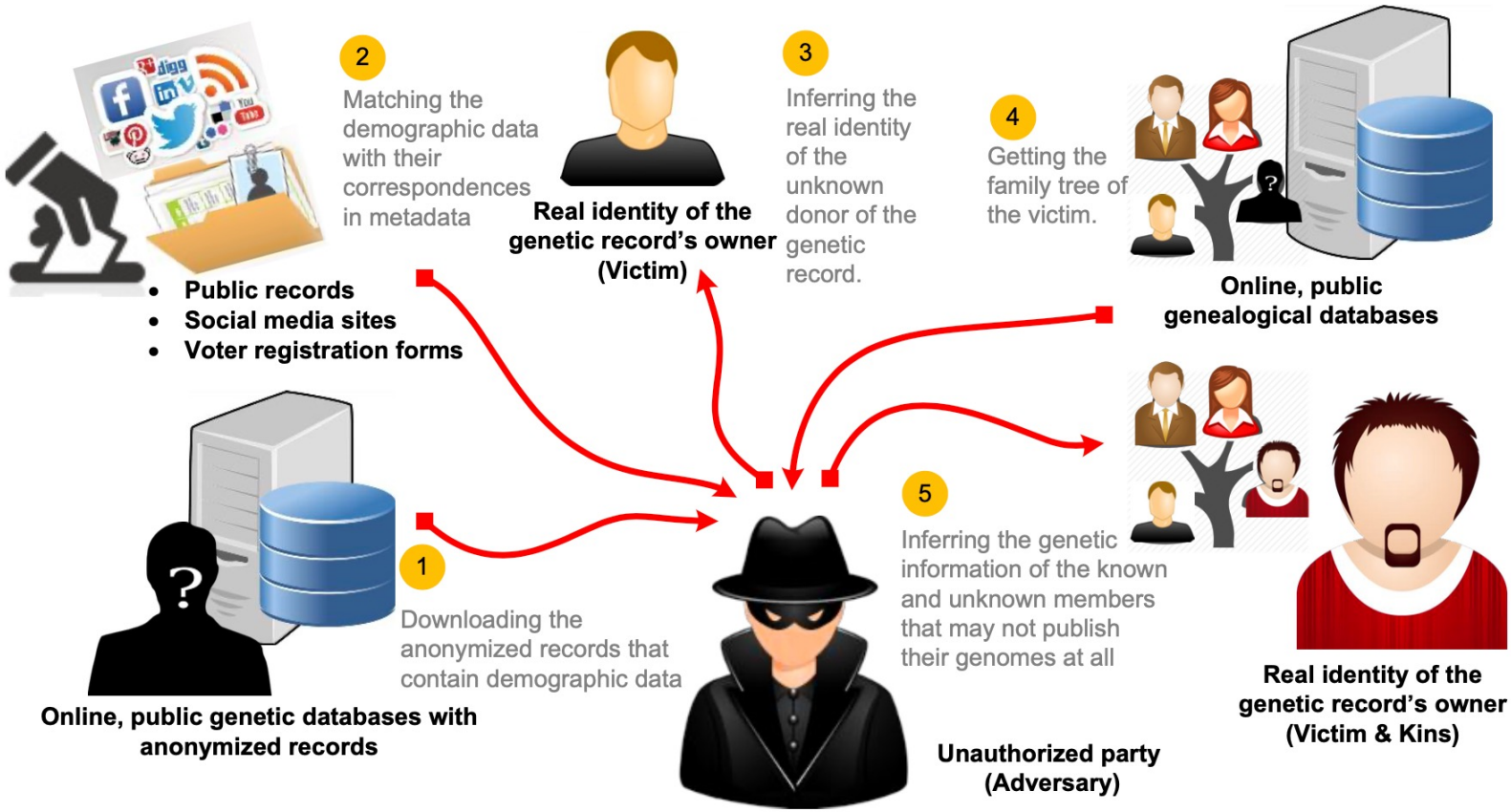


Fig. 5. A completion attack.

Alser+, "**Can you really anonymize the donors of genomic data in today's digital world?**" *10th International Workshop on Data Privacy Management (DPM)*, 2015.

Can you Really Anonymize the Donors?

(Position Paper) Can You Really Anonymize the Donors of Genomic Data in Today's Digital World?

Mohammed Alser, Nour Almadhoun, Azita Nouri, Can Alkan, and Erman Ayday

Computer Engineering Department, Bilkent University, 06800 Bilkent, Ankara, Turkey

Abstract. The rapid progress in genome sequencing technologies leads to availability of high amounts of genomic data. Accelerating the pace of biomedical breakthroughs and discoveries necessitates not only collecting millions of genetic samples but also granting open access to genetic databases. However, one growing concern is the ability to protect the privacy of sensitive information and its owner. In this work, we survey a wide spectrum of cross-layer privacy breaching strategies to human genomic data (using both public genomic databases and other public non-genomic data). We outline the principles and outcomes of each technique, and assess its technological complexity and maturation. We then review potential privacy-preserving countermeasure mechanisms for each threat.

Keywords: Genomics, Privacy, Bioinformatics

Alser+, "Can you really anonymize the donors of genomic data in today's digital world?" *10th International Workshop on Data Privacy Management (DPM)*, 2015.

Privacy-Preserving DNA Test

Our DNA Test, Reports, and Technology

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A genetic test that decodes 100% of your DNA with extremely high accuracy. 100x Whole Genome Sequencing is recommended for the discovery of rare genetic mutations.

Get Sequenced

Rapid Surveillance of Disease Outbreaks?

Figure 1: Deployment of the portable genome surveillance system in Guinea.

Quick+, "Real-time, portable genome sequencing for Ebola surveillance", *Nature*, 2016

Scalable SARS-CoV-2 Testing

BMJ Yale

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Comments (1)

Swab-Seq: A high-throughput platform for massively scaled up SARS-CoV-2 testing

Joshua S. Bloom, Eric M. Jones, Molly Gasperini, Nathan B. Lubock, Laila Sathe, Chetan Munugala, A. Sina Booeshaghi, Oliver F. Brandenberg, Longhua Guo, James Boocock, Scott W. Simpkins, Isabella Lin, Nathan LaPierre, Duke Hong, Yi Zhang, Gabriel Oland, Bianca Judy Choe, Sukantha Chandrasekaran, Evann E. Hilt, Manish J. Butte, Robert Damoiseaux, Aaron R. Cooper, Yi Yin, Lior Pachter, Omai B. Garner, Jonathan Flint, Eleazar Eskin, Chongyuan Luo, Sriram Kosuri, Leonid Kruglyak, Valerie A. Arboleda

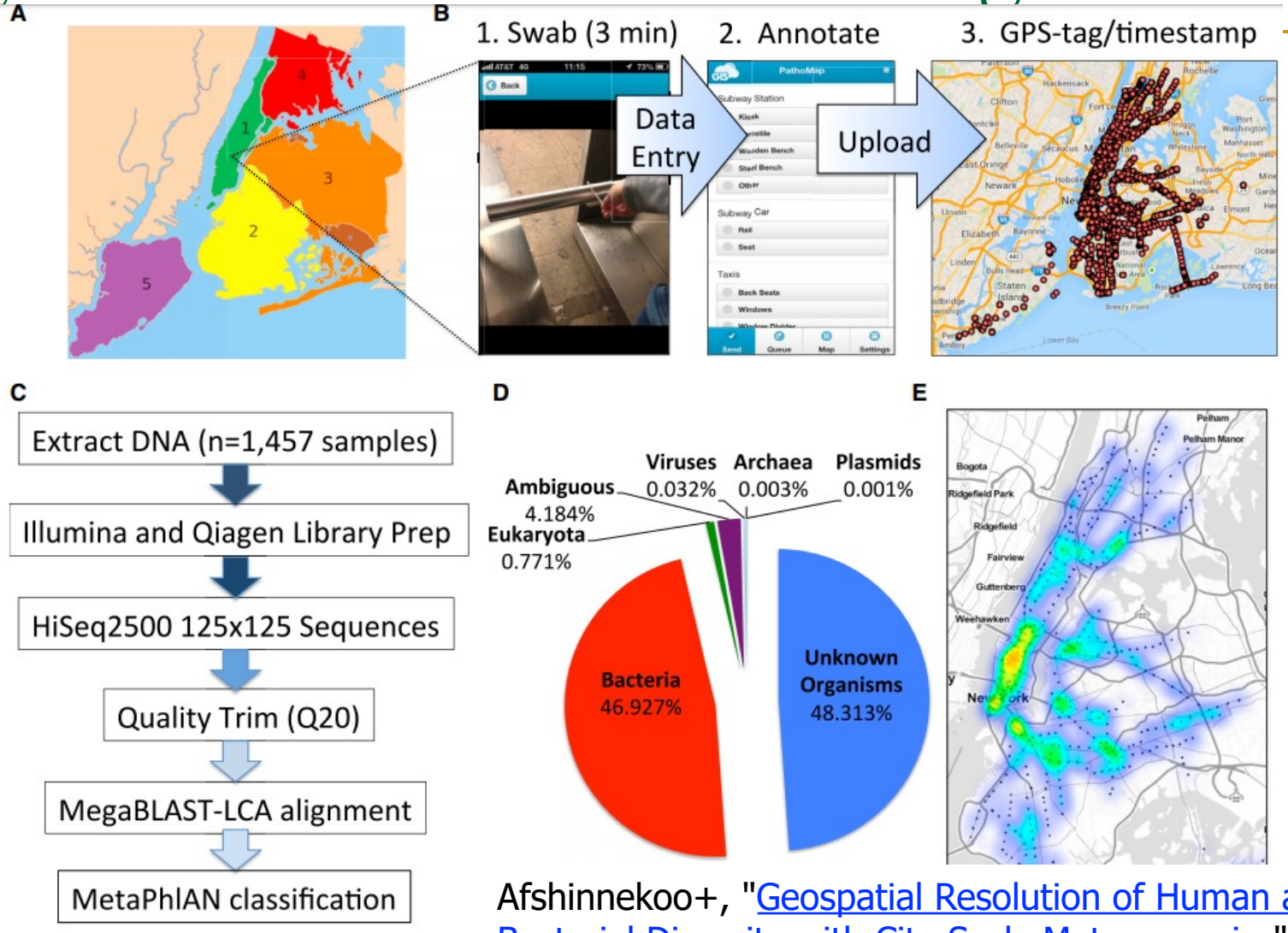
doi: https://doi.org/10.1101/2020.08.04.20167874

Bloom+, "Swab-Seq: A high-throughput platform for massively scaled up SARS-CoV-2 testing", *medRxiv*, 2020

Population-Scale Microbiome Profiling

 https://blog.wego.com/7-crowded-places-and-events-that-you-will-love/

City-Scale Microbiome Profiling



Afshinnekoo+, "Geospatial Resolution of Human and Bacterial Diversity with City-Scale Metagenomics", Cell Systems, 2015

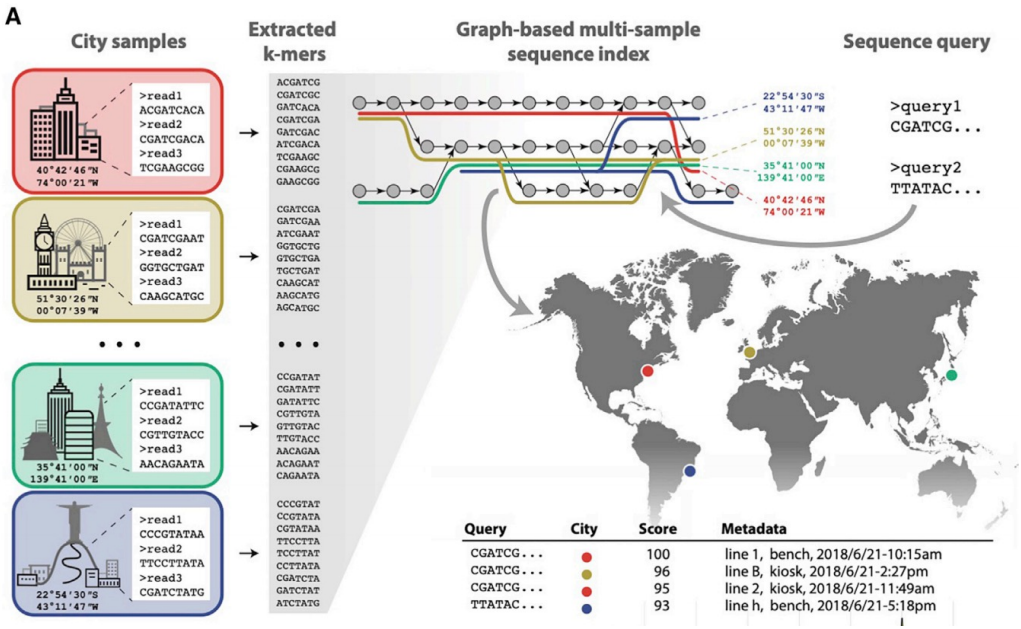
Figure 1. The Metagenome of New York City

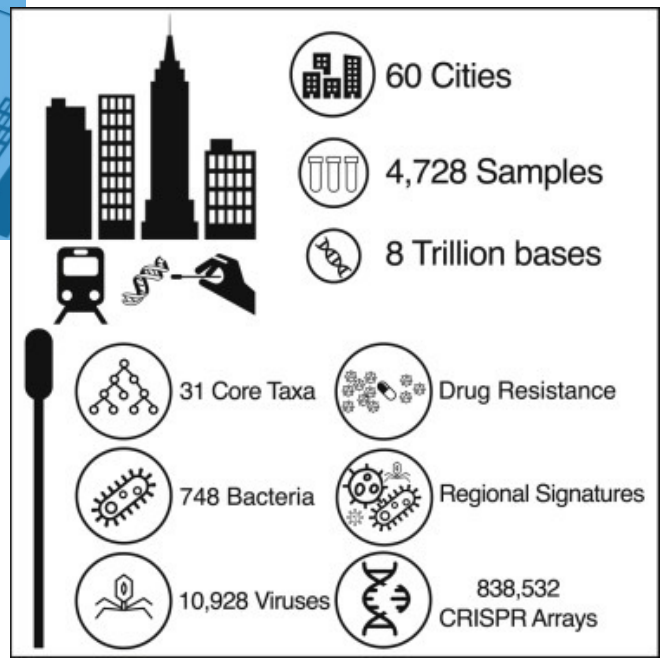
(A) The five boroughs of NYC include (1) Manhattan (green)

(B) The collection from the 466 subway stations of NYC across the 24 subway lines involved three main steps: (1) collection with Copan Elution swabs, (2) data entry into the database, and (3) uploading of the data. An image is shown of the current collection database, taken from http://pathomap.giscloud.com.

(C) Workflow for sample DNA extraction, library preparation, sequencing, quality trimming of the FASTQ files, and alignment with MegaBLAST and MetaPhlAn to discern taxa present

Population-Scale Microbiome Profiling





Danko+, "A global metagenomic map of urban microbiomes and antimicrobial resistance", Cell, 2021

Plague in New York Subway System?

Plague (Yersinia Pestis)

Harvard Health Publishing
HARVARD MEDICAL SCHOOL
Trusted advice for a healthier life

What Is It?

Published: December, 2018

Plague is caused by Yersinia pestis bacteria. It can be a life-threatening infection if not treated promptly. Plague has caused several major epidemics in Europe and Asia over the last 2,000 years. Plague has most famously been called "the Black Death" because it can cause skin sores that form black scabs. A plague epidemic in the 14th century killed more than one-third of the population of Europe within a few years. In some cities, up to 75% of the population died within days, with fever and swollen skin sores.

Plague in New York Subway System?

Plague (Yersi

What Is It?

Published: December, 2018

Plague is caused by Yersinia
treated promptly. Plague ha
last 2,000 years. Plague has
cause skin sores that form b
than one-third of the popul
the population died within

The New York Times

Bubonic Plague in the Subway System? Don't Worry About It

In October, riders were not deterred after reports that an Ebola-infected man had ridden the subway just before he fell ill. Robert Stolarik for The New York Times

he
re
of

https://www.nytimes.com/2015/02/07/nyregion/bubonic-plague-in-the-subway-system-dont-worry-about-it.html

The findings of Yersinia Pestis in the subway received wide coverage in the lay press, causing some alarm among New York residents

Failure of Bioinformatics

data. Rob Knight, a professor in the department of pediatrics at the University of California, San Diego, calls this type of error "a failure of bioinformatics," in that Mason had assumed the gene fragments were unique to the pathogens, when in fact they can also be detected in other

Living in a microbial world
Charles Schmidt
Nature Biotechnology, **volume 35**, pages401–403 (2017)
https://www.nature.com/articles/nbt.3868

There is a critical need for **fast** and

accurate genome analysis.

Achieving Intelligent Genome Analysis?

How and where to enable

fast, accurate, cheap,

privacy-preserving, and exabyte scale

analysis of genomic data?

Agenda for Today

- What is Genome Analysis?
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Genome Analysis

NO machine can read the ***entire*** content of a genome

>CCTCCTCAGTGCCACCCAGCCCACTGGCAGCTCCCAAACAGGCTCTTATTAAAACACCCTGTTCCCTGCCCCTTGGAGTGAGGTGTCAAG
GACCTAAACTAAAAAAAAAAAAAGAAAAAGAAAAGAAAAAGAATTTAAAATTTAAGTAATTCTTTGAAAAAAACTAATTTCTAAGCTTCTT
CATGTCAAGGACCTAATGTGCTAAACAGCACTTTTTTGACCATTATTTTGGATCTGAAAGAAATCAAGAATAAATGAAGGACTTGATACATTG
GAAGAGGAGAGTCAAGGACCTACAGAAAAAAAAAAAAAAGAAAAAGAAAAGAAAAAGAATTTAAAATTTAAGTAATTCTTTGAAAAAA
ACTAATTTCTAAGCTTCTTCATGTCAAGGACCTAATGTCTGTGTTGCAGGTCTTCTTGCATTTCCCTGTCAAAAGAAAAAGAATTTAAAATTT
AAGTAATTCTTTGAAAAAAACTAATTTCTAAGCTTCTTCATGTCAAGGACCTAATGTCAGGCCAAGAGTTGCAAAAAAAAAAAAAGAAAAA
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AAAAAAAAAAGAAAAAGAAAAGAAAAAGAATTTAAAATTTAAGTAATTCTTTGAAAAAAACTAATTTCTAAGCTTTTTCATGTCAAGGACC
TAATGTAGCTATACTGAACGTTATCTAGGGGAAAGATTGAAGGGGAGCTCTAAGGTCAACACACCACCACTTCCCAGAAAGCTTCTTCA.....

Genome Analysis

NO machine can read the ***entire*** content of a genome

Why?!

>CCT…CAAG
GACC…TCTT
CATGT…CATTG
GAAG…AAAA
ACTA…ATTT
AAGT…AAAA
GAAA…ATGG
TTGTC…GAAA
AAAAAAAAAAGAAAAAGAAAAGAAAAAGAATTTAAAATTTAAGTAATTCTTTGAAAAAAACTAATTTCTAAGCTTTTTCATGTCAAGGACC
TAATGTAGCTATACTGAACGTTATCTAGGGGAAAGATTGAAGGGGAGCTCTAAGGTCAACACACCACCACTTCCCAGAAAGCTTCTTCA……

Suggested Readings

nature methods

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Published: November 2009

Next-generation sequencing library preparation: simultaneous fragmentation and tagging using *in vitro* transposition

Fraz Syed ✉, Haiying Grunenwald & Nicholas Caruccio

Nature Methods **6**, i–ii (2009) | Cite this article

16k Accesses | **4** Citations | **5** Altmetric | Metrics

https://www.nature.com/articles/nmeth.f.272

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nature biotechnology

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Published: 09 October 2008

Next-generation DNA sequencing

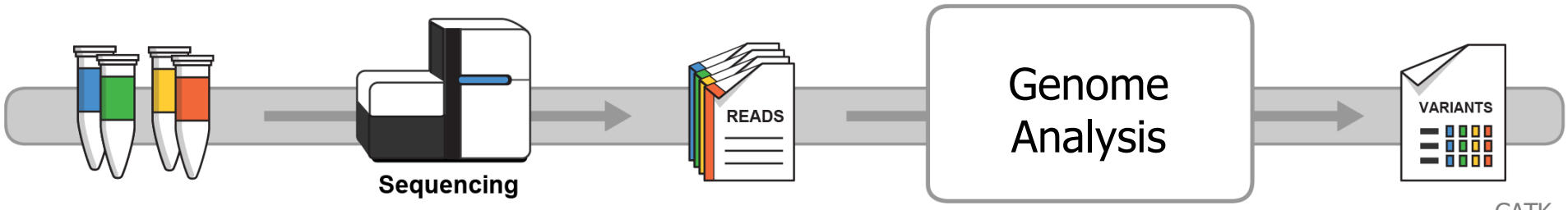
Jay Shendure ✉ & Hanlee Ji ✉

Nature Biotechnology **26**, 1135–1145 (2008) | Cite this article

149k Accesses | **2645** Citations | **79** Altmetric | Metrics

https://www.nature.com/articles/nbt1486

Genome Sequencer is a Chopper



GATK

CCCCCCTATATATACGTACTAGTACGT
ACGACTTTAGTACGTACGT
TATATATACGTACTAGTACGT
ACGTACG CCCCTACGTA
TATATATACGTACTAGTACGT
ACGACTTTAGTACGTACGT
TATATATACGTACTAAAGTACGT
TATATATACGTACTAGTACGT
ACG TTTTTAAAACGTA
TATATATACGTACTAGTACGT
ACGAC GGGGAGTACGTACGT

1x10^{12} bases*

44 hours*

<1000 $

* NovaSeq 6000

High-Throughput Sequencers

Illumina MiSeq

Pacific Biosciences Sequel II

Oxford Nanopore PromethION

Oxford Nanopore MinION

Illumina NovaSeq 6000

Pacific Biosciences RS II

Oxford Nanopore SmidgION

… and more! All produce data with different properties.

Oxford Nanopore Sequencers

MinION Mk1B

MinION Mk1C

GridION Mk1

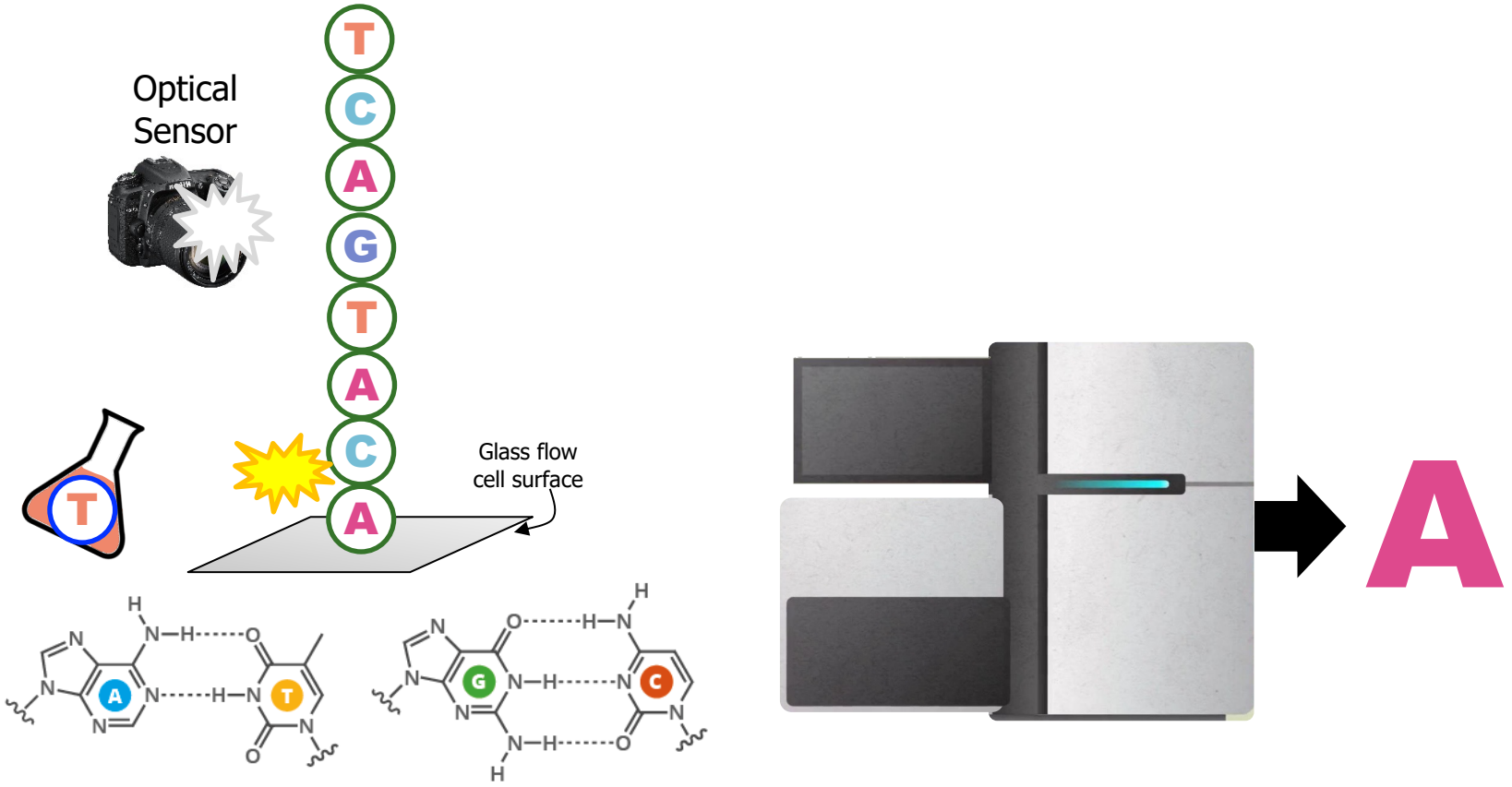
PromethION 24/48

	MinION Mk1B	MinION Mk1C	GridION Mk1	PromethION 24	PromethION 48
Read length	> 2Mb	> 2Mb	> 2Mb	> 2Mb	> 2Mb
Yield per flow cell	50 Gb	50 Gb	50 Gb	220 Gb	220 Gb
Number of flow cells per device	1	1	5	24	48
Yield per device	<50 Gb	<50 Gb	<250 Gb	<5.2 Tb	<10.5 Tb
Starting price	$1,000	$4,990	$49,995	$195,455	$327,455

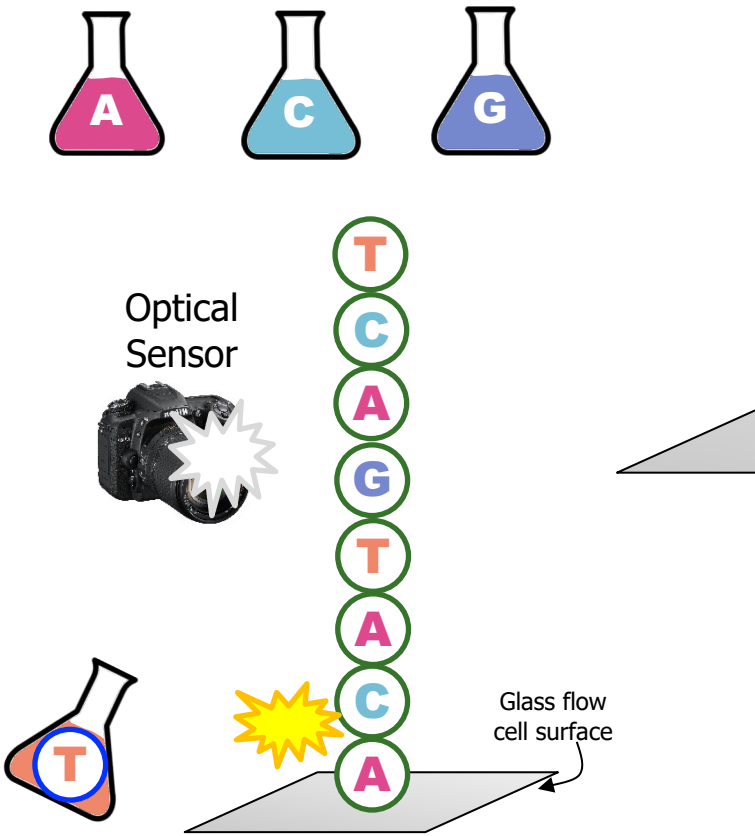
Illumina Sequencers

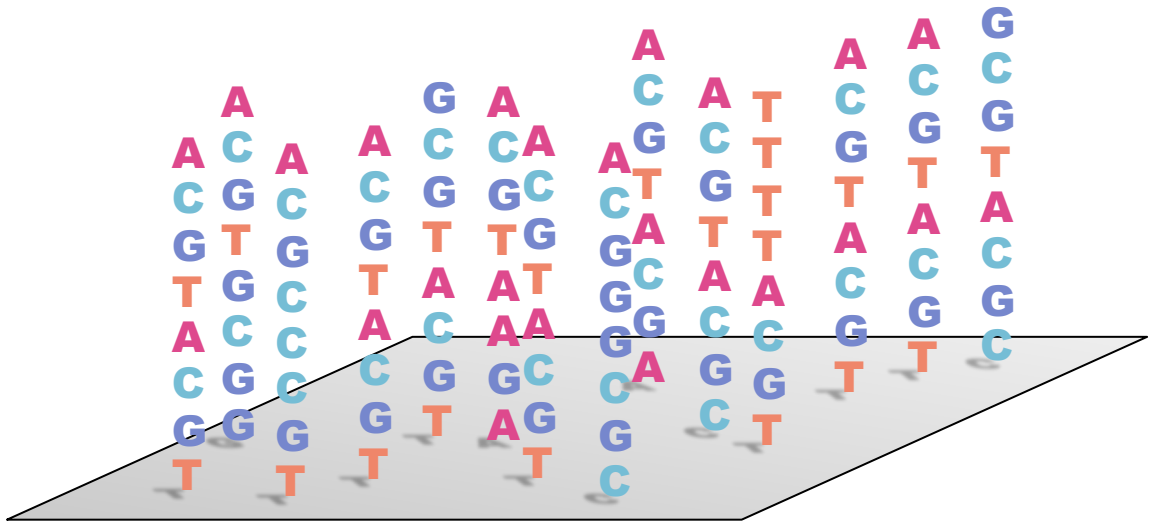
Run time	9.5–19 hrs	4–24 hrs	4–55 hrs	12–30 hrs	24-48 hrs	13-44 hrs
Max. reads per run	4 million	25 million	25 million	400 million	1 billion	20 billion
Max. read length	2 × 150 bp	2 × 150 bp	2 × 300 bp	2 × 150 bp	2 × 150 bp	2 x 250
Max. output	1.2 Gb	7.5 Gb	15 Gb	120 Gb	300 Gb	6000 Gb
Estimated price	$19,900	$49,500	$128,000	$275,000	$335,000	$985,000

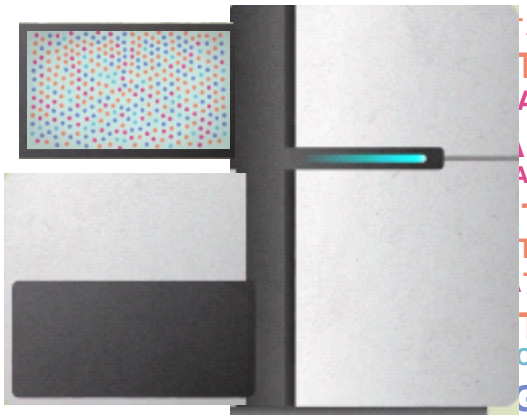
How Does Illumina Machine Work?



How Does Illumina Machine Work?



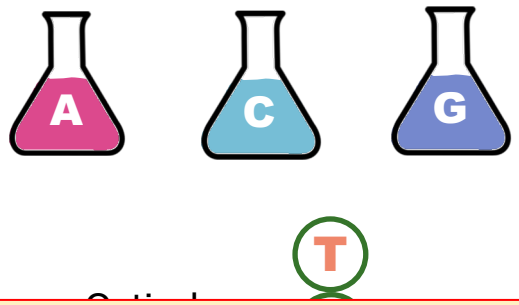




Billions of Short Reads

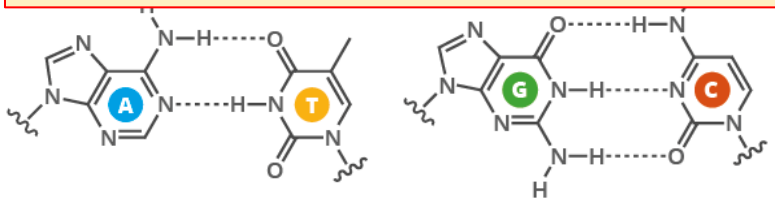
DNA fragment = Read

How Does Illumina Machine Work?



Check Illumina virtual tour:

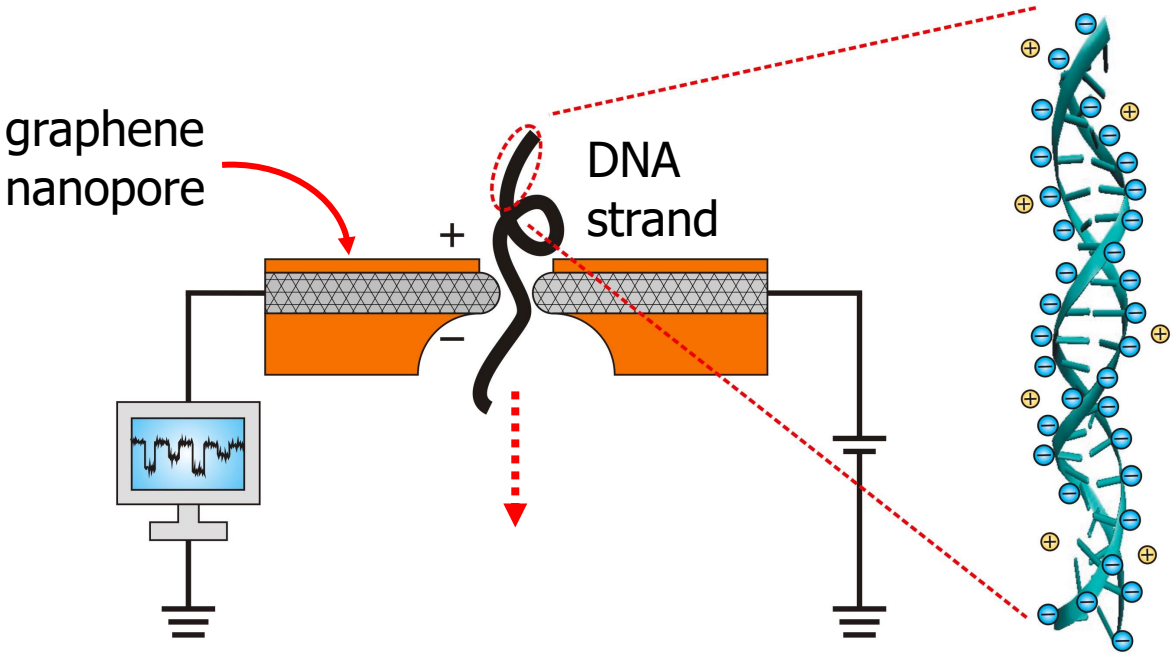
https://emea.illumina.com/systems/sequencing-platforms/iseq/tour.html



TTTAAAACGTA
CGTACTAGTACGT
GGGAGTACGTACGT

DNA fragment = Read

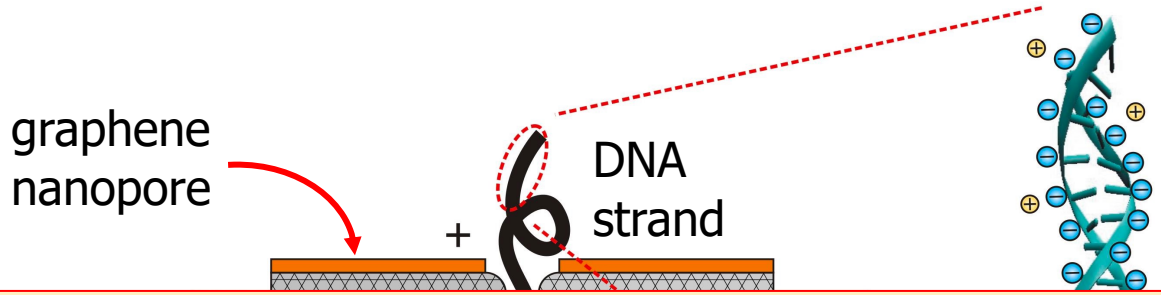
How Does Nanopore Machine Work?



- **Nanopore** is a nano-scale hole (<20nm).
- In nanopore sequencers, an **ionic current** passes through the nanopores
- When the DNA strand passes through the nanopore, the sequencer measures the the **change in current**
- This change is used to identify the bases in the strand with the help of **different electrochemical structures** of the different bases

Figure is adapted from: https://phys.org/news/2013-12-gene-sequencing-future.html

How Does Nanopore Machine Work?



Check Nanopore virtual tour:

https://nanoporetech.com/resource-centre/minion-video

- measures the the **change in current**
- This change is used to identify the bases in the strand with the help of **different electrochemical structures** of the different bases

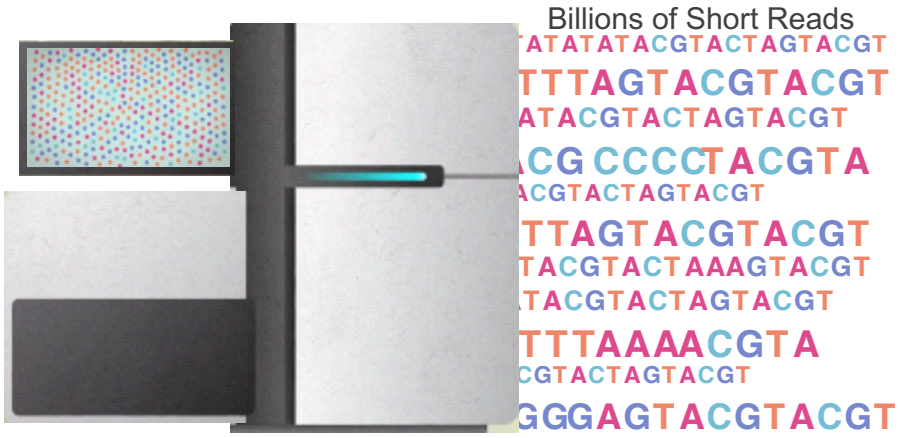
Figure is adapted from: https://phys.org/news/2013-12-gene-sequencing-future.html

Common Disadvantages!

Regardless the sequencing machine,

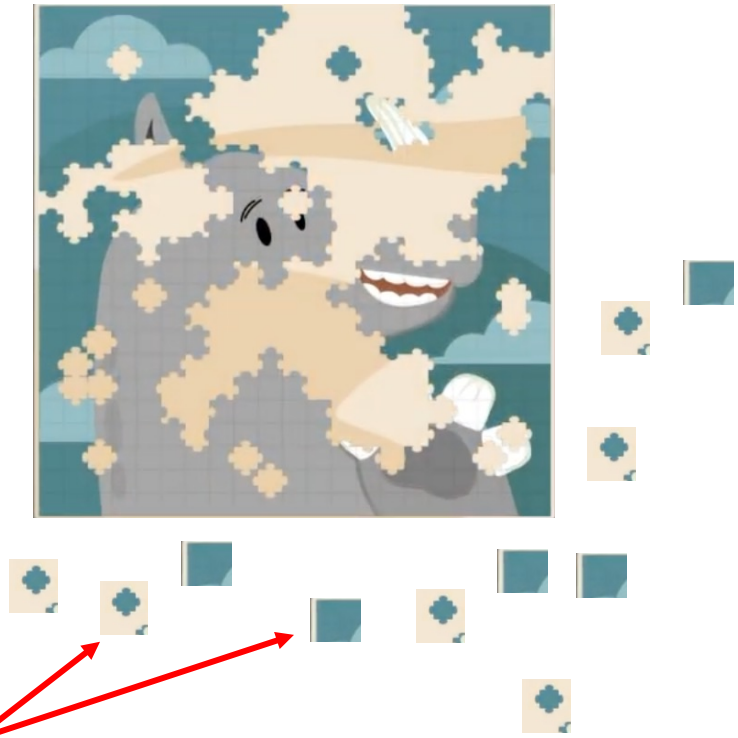
reads still lack information about their order and location

(which part of genome they are originated from)



Solving the Puzzle

Reference genome



Reads

https://www.pacb.com/smrt-science/smrt-sequencing/hifi-reads-for-highly-accurate-long-read-sequencing/

HTS Sequencing Output

Small pieces of a puzzle
short reads (Illumina)

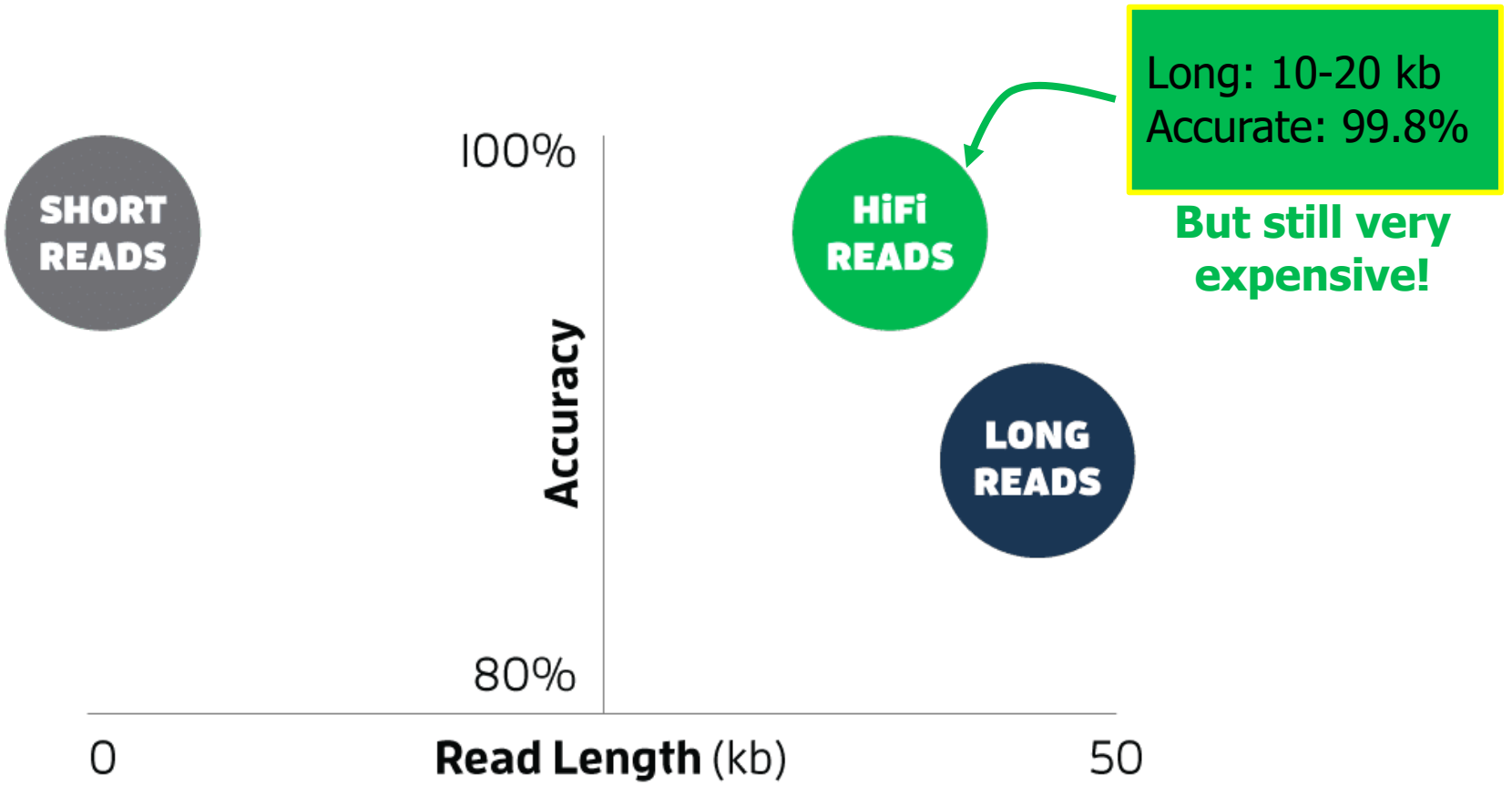
Large pieces of a puzzle
long reads (ONT & PacBio)

Which sequencing technology is the best?

- ❑ 100-300 bp
- ❑ 500-2M bp
- ❑ low error rate (~0.1%)
- ❑ high error rate (~15%)

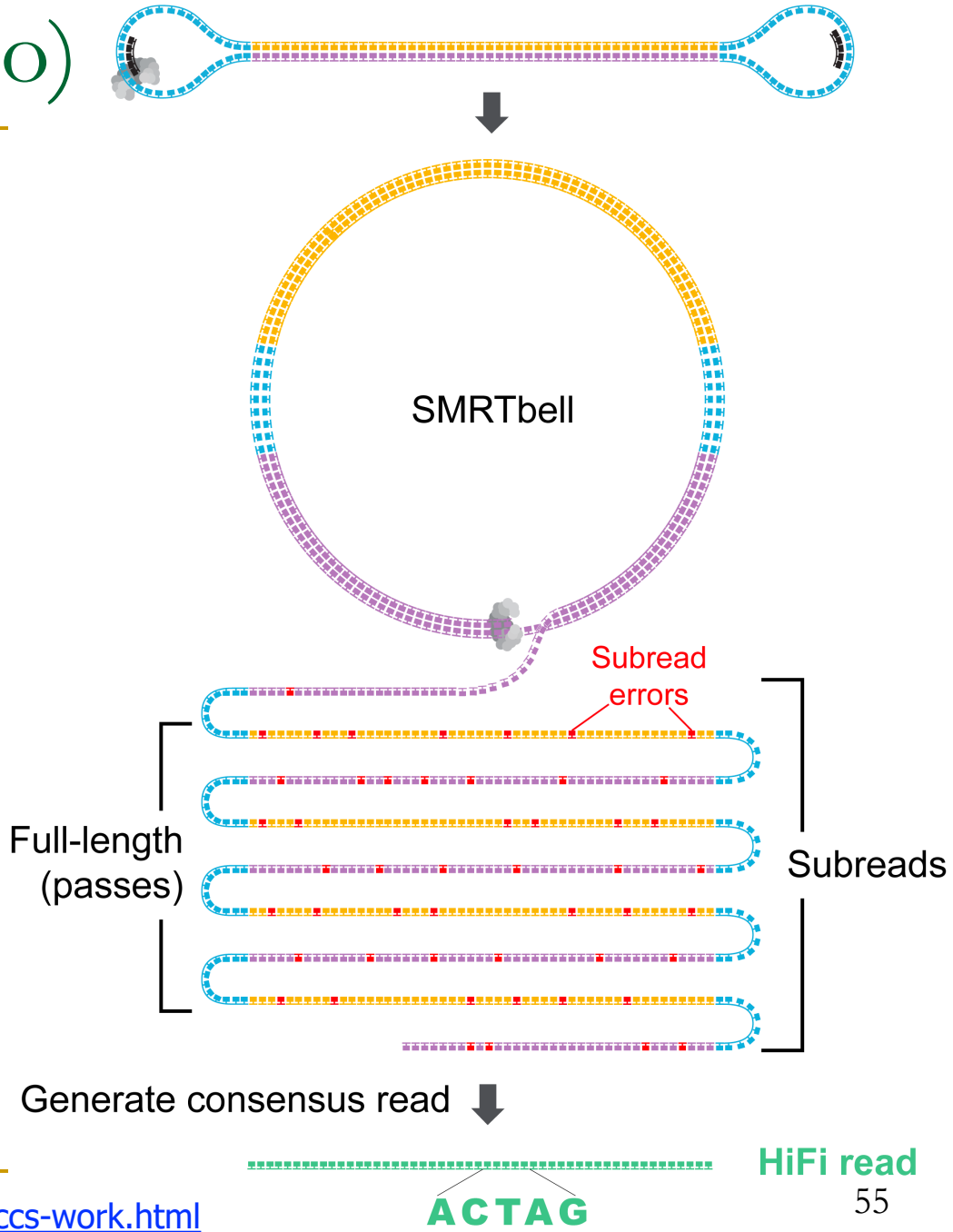
https://www.pacb.com/smrt-science/smrt-sequencing/hifi-reads-for-highly-accurate-long-read-sequencing/

HiFi Reads (PacBio)



Wenger+, "Accurate circular consensus long-read sequencing improves variant detection and assembly of a human genome", *Nature Biotechnology*, 2019

HiFi Reads (PacBio)



https://ccs.how/how-does-ccs-work.html

Changes in sequencing technologies can render some read mapping algorithms irrelevant

Read Mapping in 111 pages!

In-depth analysis of 107 read mappers (1988-2020)

Mohammed Alser, Jeremy Rotman, Dhrithi Deshpande, Kodi Taraszka, Huwenbo Shi, Pelin Icer Baykal, Harry Taegyun Yang, Victor Xue, Sergey Knyazev, Benjamin D. Singer, Brunilda Balliu, David Koslicki, Pavel Skums, Alex Zelikovsky, Can Alkan, Onur Mutlu, Serghei Mangul
"Technology dictates algorithms: Recent developments in read alignment"
Genome Biology, 2021
[Source code]

Alser *et al. Genome Biology* (2021) 22:249
https://doi.org/10.1186/s13059-021-02443-7

Genome Biology

REVIEW Open Access

Technology dictates algorithms: recent developments in read alignment

Mohammed Alser[1,2,3†], Jeremy Rotman[4†], Dhrithi Deshpande[5], Kodi Taraszka[4], Huwenbo Shi[6,7], Pelin Icer Baykal[8], Harry Taegyun Yang[4,9], Victor Xue[4], Sergey Knyazev[8], Benjamin D. Singer[10,11,12], Brunilda Balliu[13], David Koslicki[14,15,16], Pavel Skums[8], Alex Zelikovsky[8,17], Can Alkan[2,18], Onur Mutlu[1,2,3†] and Serghei Mangul[5*†]

Feedback From Our Community!

James Ferguson
@Psy_Fer_

This is awesome! I've got my evening reading sorted.

Stéphane Le Crom
@slecrom

Very complete article on the evolution of read alignment algorithms. #NGS #genomics

Svetlana Gorokhova
@SGorokhova

An impressive overview of read alignment methods over the last three decades

BContrerasMoreira @BrunoContrerasM · Sep 10

Replying to @mealser @GenomeBiology and 3 others

Buen hilo de repaso sobre la evolución de los algoritmos de alineamiento de secuencias a medida que ha mejorado la tecnología de secuenciación

https://twitter.com/mealser/status/1435223377644503040

Looking forward,

Will we be able to read

the entire genome sequence?

P&S Accelerating Genomics

Lecture 3:
Introduction to Sequencing

Dr. Mohammed Alser


@mealser

ETH Zurich

Fall 2022

3 November 2022

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ETH zürich